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ON THE STRUCTURE OF CROTOCIN AN ANTIFUNGAL ANTIBIOTIC

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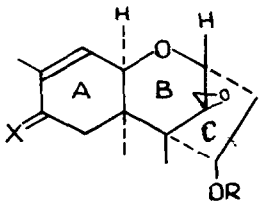
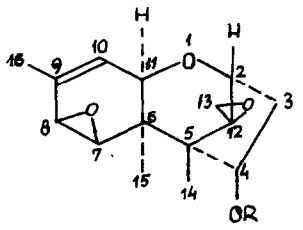
CROTOCIN, $C_{19}H_{24}O_5$ (antibiotic T)[1,2] isolated in Hungary from a new Cephalosporium strain, named Cephalosporium crotocinigenum sp. nov.[3] belongs to the group of sesquiterpenoid-ester antibiotics, members of which are trichothecin[4], verrucarines and roridines[5], trichodermin[6] and diacetoxy-scirpenol[7]. All of them are active against a number of pathogenic fungi and inhibit in low concentrations the growth of various cell types in tissue culture. Crotocin III[8,9] was found to be an ester of isocrotonic acid, similarly to trichothecin I. The alcohol components of trichothecin and crotocin, trichothecolone II and crotocol IV respectively are isomeric but not identical. In the following we are going to submit chemical and spectroscopic evidence in favour of the proposed structures III and IV for crotocin and crotocol.





I trichothecin X = O, R = $-OO-CH=CH-CH_3$

II trichothecolone X = O, R = H

XVII trichodermol X = H_2, R = H

III crotocin

IV crotocol

The IR spectra of the two esters I and III are very similar, crotocin however has no band at 1686 cm^{-1} and therefore contains no α,β unsaturated keto group. It also does not form a 2:4-dinitrophenylhydrazone in contrary to trichothecine.

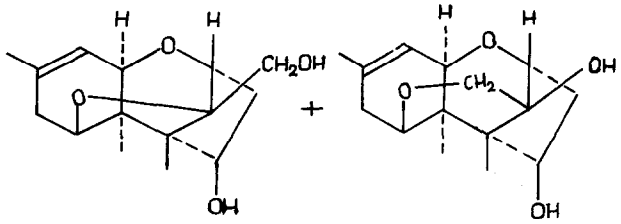
Crotocol, $C_{15}H_{20}O_4$, a readily crystallizing substance, is a diepoxide alcohol IV, mp. 154°, λmax 2100 Å (ε =4830) in ethanol, $[\alpha]_D^{20}$ = -6,4° (c = 2,01 in chloroform) which is formed upon mild alkaline hydrolysis of crotocin or applying the method of Zemplén[11] and on microbiological hydrolysis[12]. X-ray diffraction data obtained from crotocol crystals lead to an orthorombic unit cell. Results are consistent with the $P2_12_12_1$ space group[10].

Kuhn-Roth determinations showed that crotocol must posses three C-methyl groups. No methoxyl group was detected. It contains one active hydrogen (hydroxyl group) and consequently gives readily an acetyl derivative, mp. 128° V. The latter on hydrolysis with methanolic potassium hydroxyde gave crotocol. The band at 1670 cm^{-1} in the IR spectrum of crotocol is attributed to ethylenic unsaturation of the type RR'>C=CHR". Crotocol produced OH ions even at room temperature with sodium thiosulphate in 50% acetone[13], a typical reaction of epoxides.

Crotocol was readily hydrogenated at ordinary pressure and room temperature with palladium-charcoal catalyst in methanol, one mol of hydrogen being rapidly absorbed to give a crystalline mixture of dihydroisocrotocol-A VI and dihydroisocrotocol-B VII respectively. During this process a new hydroxyl group - but no new methyl group (NMR) was formed in both products and the reaction of epoxides disappeared. The olefinic double bond of crotocol was retained and could be saturated only after 1-2 hours. Reaction of crotocol with $LiAlH_4$ gave only compound B VII. By comparison, the single epoxide ring of trichothecolone was not hydrogenolized catalytically under similar conditions, the ethylenic double bond being only saturated. Trichodermol[6] reacts similarly to trichothecolone. Furthermore, treatment of verrucarol with $LiAlH_4$ gave a dihydroverrucarol contąining a tertiary methyl group more than the starting material[5].

The enhanced reactivity of crotocol on catalytic hydrogenation can be explained by a sterically favourably sited diepoxide system, which is confirmed by the Stuart-Briegleb stereomodel of crotocol. Similar results are mentioned by Agnello, Pinson jr. and Laubach[14]. On catalytic hydrogenation of 8α(14α), 9α(11α)-diepoxido-6,22-ergostadien-3β-ol acetate over Lindlar catalyst at ordinary pressure and room temperature one mol of H_2 is absorbed. The resulting product is 6,8,22-ergostatrien, 3β, 11α, 14α triol-3-acetate i.e. the diepoxide system is split and two new hydroxyl groups are formed together with a new olefinic double bond. Crotocol on catalytic hydrogenation over Lindlar catalyst similarly took up one mol of hydrogen. The resulting mixture consisted of dihydroisocrotocol-A and B for which structures VI and VII are now proposed and will be discussed later.

crotocol IV + H_2 ⟶



dihydroisocrotocol-A VI

dihydroisocrotocol-B VII

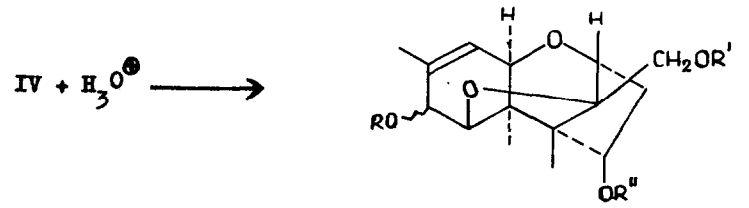
Dihydroisocrotocol-A and B can be separated by thin layer chromatography. VI gives an amorphous diacetate VIII, upon acetylation at room temperature in pyridine-acetic anhydride. VI gives also a crystalline trityl ether IX, which can also be obtained on hydrogenation of crotocin, followed by tritylation and alkaline hydrolysis. On oxydation with chromic anhydride, VI gave the corresponding ketonic acid $C_{15}H_{18}O_5$ X. (mp. 178-181°) but was recovered unchanged upon treatment with both aqueous H_2SO_4 and $LiAlH_4$.

These reactions indicate clearly the presence of a primary alcoholic function.

Dihydroisocrotocol B was recovered unchanged upon treatment with concentrated HCl at room temperature but gave a crystalline monoacetate XI upon treatment at 20° with acetic anhydride and pyridine indicating the presence of a

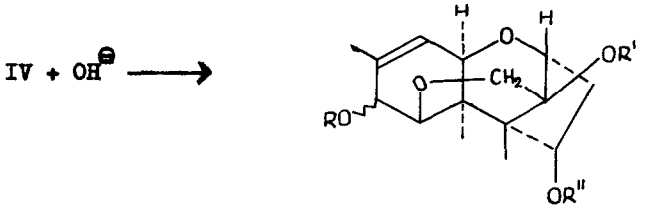
tertiary hydroxyl-group which resists acetylation.

Crotocol is extremely sensitive toward acids; 0,02 N-sulphuric acid or 0,5 N-acetic acid at room temperature give isocrotocol A XII, mp. 227°, containing three OH groups which upon acetylation at 20° gave a crystalline triacetate XIII, melting at 122°. XII gives also a mono-trityl ether XIV.



XII. R = R' = H=R" XIII. R = R' = R" = $OOCH_3$ XIV. R = R"= H; R' = Trityl

When crotocol and 5 % sodium hydroxide solution were boiled, a crystalline material, isocrotocol B XV, mp. 230°, was isolated, which on acetylation at 20° gave a diacetate XVI, mp. 158°, a tertiary hydroxyl group remaining free.



XV. R = R' = R" = H XVI. R' = H; R = R" = $OOCH_3$

In the following we will discuss the NMR spectra of crotocol and its derivatives which provide strong evidence for the proposed structures IV, VI, VII and XV. The spectra were obtained at 60 and/or 100 MHz from $CDCl_3$ solutions containing TMS as internal reference and extensive use was made of the double resonance technique[15] for the correct assignment of chemical shifts and the detection of small and unusual couplings. The results are given in Table 1. Chemical shifts are in p.p.m. from TMS, coupling constants (absolute value) in Hz.

A first look at table no. 1 shows immediately some striking similarities between the spectra of crotocol IV, trichodermol XVII and trichothecolone II. Each compound has two tertiary methyl groups (δ = 0,75 - 1.0) and a methyl

Table 1.

	C_{16}	C_{15}	C_{10}	C_{11}	C_7	C_8	C_{13}	$C_{13'}$	C_2	C_3	$C_{3'}$	C_4
II.	1.8	1.0	6.49 10–16∿1.0	3.94 10.11=5.8	C_7=2.23/7,7'=17.4 C_7±2.95/7±15∿1.0		3.12 13–13'=3.75	2.81	3.90 2–3∿0.2 2–3'=6.2	2.43 3–3'=17,4	1.93 3'–4=3.5	4.35 4–3=7.9
IV.	1.99	1.01	5.70 10–16∿1.5 10– 8∿2.4	3.69 10.11=7.0 11– 7=3.4	3.38 7–8=4.75	3.13	3.18 13–13'=3.8	2.96	3.85 2–3∿0.2 2–3'=5.0	2.70 3–3'=16.0	1.93 3'–4=3.5	4.30 4–3=7.0
V.	1.98	0.93	5.75 10–16∿1.5 10– 8∿2.4	3.78 10–11=7.0 11– 7=3.4	3.37 7–8=4.75	3.14	3.18 13–13'=3.6	2.96	3.90 2–3∿0.2 2–3'=4.5	2.48 3–3'=15.7	2.05 3'–4=5.5	5.32 4–3=8.5
VI.	1.75	1.06	5.65 10–16∿1.0	4.25 10–11=5.5 11– 7=1.0	3.61	2.15 – CH_2 – 8–10∿1.2	3.73 13–13'=11.0	3.67	3.98 2–3∿0.2 2–3'=5.0	2.40 3–3'=15.8	2.05 3'–4=5.0	4.0 4–3=8.5
VII.	1.74	1.18	5.49 10–16∿1.5 10– 8=2.0	3.53 10–11=6.0 11– 7=2.5	3.92 7–8∿3.0	2.20 –CH_2 –	3.96 13–13'=12.0	3.67	4.28 2–3<0.2 2.3'=5.1	2.45 3–3'=15.5	2.08 3'–4=3.5	4.25 4–3=7.5
VIII.	1.78	0.96	5.72	4.23?	3.5–4.6 Region	2.25 –CH_2 –	–3.5–4.6 Region		———	2.47 3–3'=18.0	1.95	5.23(?) 4–3=9.0
X.	1.82	1.17	5.75 10–16∿1.5	4.42 10–11=6.1 11– 7∿2.0	3.88	2.25 –CH_2 –	–	–	4.45 2–3∿0.2 2–3=5.2	2.7 3–3'=20.0	3.2	–
XI.	1.74	1.05	∿5.50 10–16∿1.5	3.64 10–11=6.0 11– 7=2.5	3.86 7–8=3.0	2.20 –CH_2 –	4.01 13–13'=12.0	3.64	4.27 2–3<0.2 2–3'=5.0	2.39 3–3'=15.0	∿2.16 3'–4=5.0	5.53 4–3=8.0
XIII.	1.73	0.98	5.93 10–16∿1.0 10– 8=2.5	3.85 11–10=5.6 11– 7=2.0	4.05 7–8<0.5	5.67	4.29 13–13'=11.3	4.13	4.1 2–3∿0.2 2–3'=6.3	2.47 3–3'=16.3	1.95 3'–4=5.7	5.26 4–3=8.5
XVI.	1.73	1.00	5.72	3.68	3.76 7–8∿1.0	5.18	3.97 13–13'=12.0	3.66	4.23 2–3<0.2 2–3'=5.0	2.41 3–3'=16.0	∿2.12 3'–4=4.5	5.51 4–3=8.0
XVII.	1.70	0.82	5.41 10–16∿1.0	3.51 10–11=5.5	1.35 –CH_2–	2.0 –CH_2 –	∿3.1 13–13'=4.0	2.8	3.81 2–3<0.2 2–3'=5.0	2.60 3–3'=15.5	∿1.9 3'–4=3.5	4.32 4–3=8.0

group on a double bond (δ = 1,7 - 1.99). The resonances for the protons at C_2, C_3, C_4 and C_{13} are essentially identical with respect to chemical shifts and coupling constants and are strong evidence for the presence of the C_{12}-C_{13} epoxide in crotocol.

A major difference between crotocol and trichothecolone is found in the resonance of the protons at C_7, C_8, and C_{10}. In fact, where the two protons at C_7 in trichothecolone give an AB system with a typical geminal coupling constant of J_{AB} = 17.4 Hz, in crotocol we observe two vicinal protons at C_7 and C_8 with δ H_7 = 3,38, δ H_8 = 3.13 and J_{7-8} = 4.75 Hz. The chemical shifts and coupling constants fit nicely with the observed resonances for two vicinal protons on an epoxide ring in conjugation with a π electron system (1,2-epoxy--3-butanone gives δ_B = 3.0, δ_X = 3.32, J_{BX} = 5.2 Hz[16]). Also the resonance of H_{10} in trichothecolone (δ = 6.49) is typical for a β proton in a cyclic $\alpha\beta$-unsaturated carbonyl system - the analogous proton in carvone resonates at δ = 6.75[17] - whereas H_{10} of crotocol is found at δ = 5.70.

These facts together with the observation of an allylic coupling between H_{10} and H_8 (J_{8-10} = 2.4 Hz) and of a large long range coupling between H_{11} and H_7 ($^4J_{7-11}$ = 3.4 Hz) for which a rigorous sterical relationship is required[18], has led us to postulate the presence of a second epoxide ring in crotocol located in β configuration on the C_7 - C_8 carbon atoms.

The NMR spectra allowed a straight forward interpretation of the transformations incurred by crotocol under the influence of acids, bases and reducing agents. The products so obtained can be divided into two series, the iso-A and iso-B derivatives.

The spectra clearly show that in all the derivatives the C_{12} - C_{13} epoxide ring has been opened and a new tetrahydrofuran (iso-A) or tetrahydropyran ring (iso-B) has been formed through mutual interaction of the two original epoxides.

We observe in fact a paramagnetic shift for the C_{13} protons from δ = 2.8-3.2

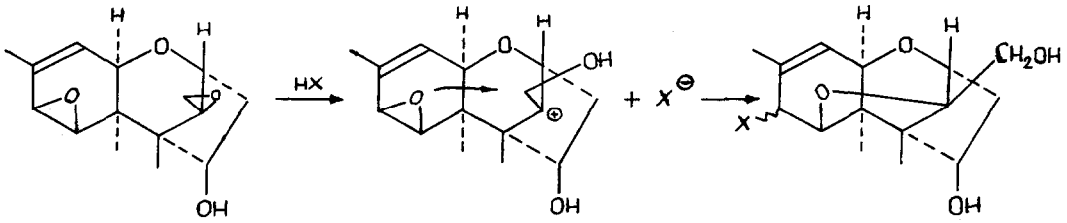
(II, IV. V. XVII) to δ = 3.64 - - 4.01 in VI, VII, XI, XVI, and to δ =4.1-4.3 in XIII. In this transformation the original geminal coupling constant $J_{13-13'}$ = 3.75 - 4.0 Hz typical for epoxides[16,19] decreases drastically to $J_{13-13'}$ = = (-) 11,0 - 12 ,0 Hz a normal value for a geminal coupling between two magnetically non equivalent protons R-CH_2-O-R' in a free group or a tetrahydropyran ring[19]. A similar paramagnetic shift is also observed for the H_2 resonance which goes from δ = 3.81 - 3.98 in II. IV. V. VI. XVII to δ = 4,1 - - 4.45 in VII, X, XI, XIII and XVI. In accordance to the proposed formulae we also observe the following features:

- in the dihydroiso-A and B series the H_8 resonance at δ = 3.13-3.14 in IV and V is lost and one finds instead two protons of a methylene group at δ = 2.15-2.25 in VI and VII and their derivatives VIII and XI which show an allylic coupling with H_{10}, J_{8-10} = 2.0-2.4 Hz. By comparison, the resonance for the C_8 methylene group of trichodermol is found at δ = 2.0
- the acetylation of VII gives XI, a monoacetate in which the H_{13} and $H_{13'}$ resonances have not been shifted; at δ =1;63 one finds a slightly broadened signal due to a tertiary hydroxyl group which disappears upon addition of D_2O to the chloroform solution
- isocrotocol A-triacetate XIII and isocrotocol B diacetate XVI show a similar behaviour as far as the C_{13} methylene resonance is concerned. XIII exhibits the paramagnetic shift to δ = 4.1-4.3 whereas XVI does not (δ = = 3.66-3.97) but gives instead a broadened signal at δ = 1.80 which disappears upon addition of D_2O. In both compounds we find a proton at C_8 in the δ = 5.67 and 5.18 region a clear indication that the newly introduced oxygen function is a secondary alcohol which undergoes easily acetylation. Sterical factors can be accounted for the difference in chemical shift between the two resonances and are also responsible for the small vicinal coupling constant between H_7 and H_8.
- during these transformations the resonances of the C_3 and C_4 protons are practically unchanged if one takes into account the normal paramagnetic shift for H_4 when going from the free alcohol to the esters in V, VIII, XI,

XIII and XVI. The same can be said of the coupling constants J_{3-4}, $J_{3-3'}$, J_{2-3} and $J_{2-3'}$, which remain unaltered in all compounds;

- the paramagnetic shift of H_2 ($\Delta\delta = 0{,}3$) upon opening of the epoxide ring at C_{12}-C_{13} is in good agreement with the observed anisotropic effects of three membered rings[20].

A characteristic feature of the chemistry of trichothecolone and its derivatives is the fact that the epoxide under certain circumstances is susceptible to intramolecular nucleophilic attacks accompanied by skeletal rearrangements[5]. Trichothecolone, verrucarol, trichodermol and their esters are rapidly attacked by strong acids with opening of the oxirane ring. During this process the protonated epoxide is attacked by the O atom of the middle ring (B) and a rearrangement takes place. In the case of crotocin or crotocol the oxirane ring mentioned above is easier attacked by the O atom of the other epoxide (between C_7 and C_8) than by the O atom of the less reactive tetrahydropyran ring (B). According to this the following reaction takes place:



with formation of a new heterocyclic ring from the two epoxide groups.

On treatment of crotocol with boiling dilute sodium hydroxide solution, the epoxide of ring A is opened and an oxygen anion attacks the other epoxide group /XV from IV/.

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